International Journal of Agricultural Science and Research (IJASR)
ISSN (P): 2250-0057; ISSN (E): 2321-0087
Vol. 9, Issue 3, Jun 2019, 333-340

NANO EMULSION SEED INVIGOURATION FOR IMPROVED GERMINATION AND SEEDLING VIGOUR IN MAIZE

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ABSTRACT

An experiment was carried out with the objective of to synthesize a novel nano-emulsion using methyl cellulose (biodegradable polymer), vitamin E and surfactant Tween 80. The emulsion was prepared with high pressure homogenization under 10000, 20000, 30000 and 40000 bars and characterized for its properties using Particle Size and Analyzer (PSA), Scanning electron microscope (SEM) and Transmission electron microscope (TEM). Seeds of Maize cv. COHM 6 were invigourated with methyl cellulose nano emulsion at different concentrations of 10, 15, 20, 25, 30, 35 and 40 ml kg^{-1} of seed and tested for its quality under in vitro conditions. The results of PSA showed that the nano emulsion developed at 40000 bars found to register the required particle size of 157.5nm. The SEM and TEM images revealed that methyl cellulose nano emulsion had core shell like micelle. The results of seed quality testing exhibited that nano emulsion coated seeds recorded higher rate of imbibitions, speed of emergence, germination and vigour index.

KEYWORDS: Nano Formulation, Methyl cellulose, Seed Encapsulation & Seed Quality

Received: Apr 27, 2019; **Accepted**: May 17, 2019; **Published**: Jun 10, 2019; **Paper Id.**: IJASRJUN201946

Original Article

INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile crops mostly grown under rainfed ecosystem. Rainfall, a critical factor in rainfed agriculture, where success depends on the management of every drop of rainfall. Pre monsoon sowing is the proven technique for utilizing the earliest opportunities of rainfall and expedite length of growing period. Farmers hesitate to adopt pre monsoon sowing because of the in consistency of onset and distribution of monsoon. Dry seeding cause damage to seeds due to higher soil temperature and moisture stress when they remain in soil for extended period, in delayed onset of monsoon situations. Hence, keeping seeds in viable conditions in such situations will helpful for the success of dry seeding. Seed coating with polymeric materials which resist heat transfer under high soil temperature and remain porous for water on the receipt of rainfall will be a good strategy for exploiting the potential of dry seeding. Good germination and emergence of healthy seeding will spell for good crop stand (Raja and Satish, 2018). There are pre-sowing seed treatments such as seed hardening, soaking of maize seeds in inorganic salts and plant growth regulators (Sakthivel, 2016) for improving the seed germination and vigour of crops under abiotic stress. Though these technologies have found effective, still there are some practical difficulties exist in adopting at filed level, which in turn focus to develop an alternate technology.

Nano science is an emerging field and is highly need in agriculture. It's important in Seed Science is recent and some of the work done in United States of America clearly demonstrates that nanoparticles can be used to

improve the seed germination in tomato (Khodakovskaya *et al*., 2009). Several metal oxide nanoparticles have been tested and found effective in seed invigoration of a wide array of crops (Sridhar, 2012; Krishnashyla, 2014; Dileep kumar, 2016, Raja *et al*., 2019). Ground seeds coated with IAA fortified Carboxymethyl cellulose nanoemulsion resulted higher rate of imbibition, germination and seedling vigour under *in vitro* conditions (Tamilarasan *et al*., 2018). This literature data clearly demonstrated that nanotechnology is a powerful tool to infuse required inputs in seeds that can facilitate improved germination regardless of environmental constraints. Hence, it is hypothesized to synthesize a nanoemulsion as seed invigorant to improve seed germination and seedling vigour of maize against abiotic stress.

MATERIALS AND METHODS

The present research was conducted at the department of Nano Science and Technology and Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, India. Vitamin (vitamin E (alpha-tocopherol)), Polymer (methylcellulose), and surfactant (tween80) were purchased from M/s. Sigma Aldrich chemicals Private Limited, Bangalore, India. These seeds, vitamin and chemicals constituted as study materials for the present investigation. Freshly harvested and genetically pure seeds of maize CO 6 were obtained from department of millets, TNAU, Coimbatore.

Preparation of Methyl Cellulose Emulsion

Conventional Methyl Cellulose based vitamin E (alpha-tocopherol) mixed emulsion was prepared using surfactant Tween 80. To prepare 100 ml of vitamin E (alpha tocopherol), methyl cellulose, vitamin E and tween 80 were mixed at the ratio of 99.4:0.1:0.5. Methyl cellulose solution was taken in a beaker and kept under constant magnetic stirring and to this vitamin E solution was added slowly drop by drop. Then the surfactant was added drop by drop. This mixture solution was homogenized under room temperature at 400 rpm completely until the formulation achieved clear state. Then the conventional emulsion was subjected to high energy homogenization where the pressure ranged from 10000 bar to 40000 bar with an interval of 10000 bar (Sharma *et al.*2015). Then homogenized samples were analysed for particle size potential, SEM and TEM.

Particle Size

Particle size and distribution pattern of hormones loaded nano formulations were determined using Nanopartica SZ-100, Horiba Scientific. Five ml of ice sonicated solution from each formulation was taken and analyzed under dynamic light scattering method using 90º or 173º at 25ºC.

SEM (Scanning Electron Microscope)

Scanning Electron Microscope is a type of electron microscope that images the sample surface by scanning it with a beam of high-energy of electrons in a raster scan pattern. Samples were mounted rigidly on a specimen holder called a specimen stub and placed in the specimen chamber for imaging the sample. Scanning Electron Microscope (Quanta 250, FEI, and Netherlands) available at the Department of Nano Science and Technology, TNAU, Coimbatore was used to characterize the surface of the sample. Samples of hormones loaded nano formulations were kept over the aluminium stub and loaded in the sample holder. Sample surface was observed at different magnifications and the images were recorded.

TEM (Transmission Electron Microscope)

The TEM FEI Technai Sprit was used to analyze the morphology of the nano formulations. For TEM studies, a drop of formulation was taken from each nano formulation after ice sonication for 15 min. and placed on copper grid and

allowed to dry in vacuum for 24 h. Then the transmission electron micrographs were taken with a W-source and an ultra-high resolution pole piece.

Effect of Nanoemulsion on Seed Quality

Maize seeds were coated with methyl cellulose nano formulation at 10, 15, 20,25,30,35 and 40 ml per kg of seed. The treated seeds were tested for revealing the bio efficacy of nano emulsion. The experiment was designed in completely randomized design with three replications. Observations on coating efficiency, rate of water uptake, speed of germination (Maguire, 1962), germination per cent (ISTA, 2013), seedling length, dry matter production and vigour index (Abdul-Baki, A. A., and Anderson, J. D, 1973).

RESULTS AND DISCUSSIONS

Synthesis and Characterization of Nano Emulsion

The characterization study demonstrated that the nanoemulsion produced at 40000 bar Found to be recorded the required particle size of 157.5nm. However, the formulation prepared at low homogenization energy of 10000 bar recorded the maximum particle size of 470.0 nm. The morphology of the methyl cellulose nano formulation viewed under SEM and TEM indicated the core shell micelle (Figure. 2a & 2b). Tamilarasan *et al*. (2019) observed that IAA loaded Carboxymethyl cellulose nanoemulsion produced at 40000 bar registered the particle

The characterization study demonstrated that the nanoemulsion produced at 40000bar found to be recorded at 40000 bar have recorded the desired particle size of 157.5nm. However, the formulation prepared at low homogenization energy of 10000 bar recorded the maximum particle size of 470.0 nm.

The morphology of the methyl cellulose based nano emulsion studied under SEM, the results revealed the presence of core shell like structures of micelle in all the formulation. In methyl cellulose based vitamin E loaded nano formulation the size of core shell structure of micelle was ranged from 283.8nm to 469.9nm. The morphology of the methyl cellulose based nano emulsion studied under TEM the results revealed the presence of core shell like structures of micelle with size ranged from 142.0 nm to 551.0 nm in vitamin E loaded formulation.

Similar results was observed by Sakthivel (2016) TEM morphology of the CMC based nano formulations loaded with GA_3 (100 ppm) and IAA (100 ppm) & surfactant Tween 80 appeared as core shell like structure of micelle. The EDAX studies confirmed the presence of elements like C, N and O which are the important components of the GA_3 and IAA loaded in the nanoformulations

Effect of Methyl Cellulose Emulsion on Maize Seed Quality

Coating Efficiency

Initially, the sample was taken 10gm of control seeds, after coated with nano emulsion with the range of 10ml to 40ml of thickness per kg of seed. As the concentration of the nano emulsion increases, there was occurs a significant and gradual increase in weight of the seed from concentration of 10ml to 40ml per kg of seed. The maximum coating efficiency was recorded in 40ml kg^{-1} (8.9%), the lowest coating efficiency value recorded in 10ml per kg of seed (2.3%) (Figure 2)

Imbibition Rate

Imbibition rate was significantly influenced by methyl cellulose nano emulsion coating with different concentrations over a period of 24 hr. In the graphical representation shows the imbibitions rate was increased over the period of time. So, the vitamin based nano emulsion does not affect the imbibition rate. The highest imbibition rate was observed in 40 ml/ kg of seed (24.1%) and the lowest was observed in control (20.9%) (Figure 3)

Speed of Germination

Momentous difference was observed in speed of emergence due to methyl cellulose nano emulsion encapsulation. The maximum speed of germination (8.2%) was observed in 40ml kg^{-1}, while the uncoated seeds recorded the lowest value (7.4) (Table 1).

Germination Percentage

Germination was expressively influenced by the different concentrations of methyl cellulose nano emulsion. Among the different concentrations, 40ml, 25ml & 20ml kg^{-1} of seed recorded the maximum germination percentage of 100. Lowest germination percentage recorded in control (96%) (Table 1).

Root Length

Root length of germinated seeds was pointedly differed due to nano emulsion coating; seeds coated with nano emulsion at 40ml per kg of seed produced longest roots (22.1cm), followed by 35ml per kg of coating, which had produced the root length of 21.8cm. Whereas control has produced root of having 18.6cm (Table 1).

Shoot Length

Shoot length of germinated seeds emphatically differed due to nano emulsion encapsulation. Among the different concentrations, seeds coated with nano emulsion of 40 ml recorded highest shoot length (16.3cm), followed by 35ml per kg of seed which recorded 16.0cm. Whereas untreated seeds had produced shoot length of having 12.3cm (Table 1).

Dry Matter Production

The experimental results on Dry matter production due to methyl cellulose nano emulsion have showed more significant improvement. As the concentration of the nano emulsion increases, there is occurs a significant and gradual increase in dry matter production from concentrations of 10ml to 40ml. The seeds coated with 40ml kg^{-1} of seed recorded the highest dry matter content of 1.2505 g per 10 seedlings, followed by 35ml kg^{-1} of seed had registered the dry matter production of 1.223g per 10 seedlings. The control seeds recorded the lowest dry matter production of 0.954 g per 10 seedlings (Table 1).

Vigour Index

The experimental results on vigour index due to methyl cellulose nano emulsion have showed more significant improvement. As the thickness of the nano formulation increases, there is occurs a significant and gradual increase in vigour index from concentrations of 10ml to 40ml. formulation at 40mlkg^{-1} of seed had recorded the maximum vigour index of 3840, which was followed by 35ml kg^{-1} of seeds (3704). Whereas the control recorded the minimum value of 2966 (Table 1).

The bio efficacy test of methyl cellulose nano emulsion invigourated seeds demonstrated that seeds, coated with nano emulsion of 40ml per kg of seeds found to be superior in terms of registering higher higher imbibitions rate (24.1%), speed of germination (8.2%), germination percentage (100%), shoot length (16.3cm), root length (22.1cm), vigour index (3840) and dry matter production (1.250gm/10 seedlings), as compared to the control which recorded which registered less imbibitions rate (20.9%), speed of germination (7.4%), germination percentage (96%), shoot length (12.3cm), root length (18.6cm), vigour index (2966) and dry matter production (0.954)gm/10 seedlings.

Sakthivel *et al*. (2016) reported**,** the results of impact analysis of nanoformulations on seed quality in cotton exhibited that seeds treated with CMC based nanoformulations (nanoemulsion) loaded with GA_3 and IAA recorded higher germination and vigour index under invitro condition.

Tamilarasan *et* al. (2018) reported With respect to efficacy testing, seeds coated with CMC based GA3 and IAA loaded nano formulation at 15 ml kg-1 had excelled the other treatments in augmenting speed of germination, physiological parameters and hydrolyzing enzymes activity in both standard and sub-standard lots. The findings of the efficacy testing of nanoformulations under different moisture regimes showed that seeds coated with CMC based GA3 and IAA loaded nano formulation at 15 ml kg-1 had excelled the other treatments in augmenting speed of emergence, germination, seedling vigour, dry matter production and antioxidant enzymes activity in both optimal and sub-optimal conditions.

CONCLUSIONS

So, it was concluded that methyl cellulose nano emulsion seed invigoration recorded higher germination, seedling vigour under *invitro* condition. Here, though the higher dose of 40ml per kg of seed performances better, it had no significance with 20ml per kg of seed. Hence it is recommended to invigoration seed with 20 ml per kg of seed. For, that bio efficacy of methyl cellulose nano emulsion on seed quality to be calculated under stress conditions as well as field.

REFERENCES

1. *Abdul-Baki, A. A., & Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria 1. Crop science, 13(6), 630-633.*
2. *C.Tamilarasan, K.Raja, KS Subramanian and P.Selvaraju.2018. synthsis and development of nano formulation for hastening seed quality in Groundnut. Res. Jr. of Agril. Sci. 10(1);50-57.*
3. *C.Vanitha, & M.Kathiravan, Response Of Blackgram (Vigna Mungo. L) To Seed Bio-Fortification And Foliar Nutrition Intervention In Relation To Seed Quality And Yield Potential.*
4. *ISTA. 2013. International Rules for Seed Testing. The International Seed Testing Association, Basssersdorf, Switzerland.*
5. *K.Raja and Sathish.2018. Seed treatments for vigorous seedling establishment and improved seed yield of black gram under rice fallow condition. Legume Research 41(1): 120-125.*
6. *K.Raja, R.Sowmya, R.Sudhagar, Pon.Satyamoorthy, K.Govindaraj and K.Subramanian. 2019.Biogenic ZnO and Cu nano particles to improve seed germination quality in black gram(Vigno mungo). Vol.235:p 164-167*
7. *Khodakovskaya, M., E. Dervishi, M. Mahmood, Y. Xu, Z. Li, F. Watanabe and A. S. Biris. 2009. Carbon nanotubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. ACS Nano, 3(10): 3221–3227.*
8. *Maguire, J. D. (1962). Speed of Germination - Aid In Selection And Evaluation for Seedling Emergence And Vigor 1. Crop*

science, 2(2), 176-177.

9. *Marković, M., Josipović, M., Šoštarić, J., Jambrović, A., & Brkić, A. (2017). Response of maize (Zea mays L.) grain yield and yield components to irrigation and nitrogen fertilization. Journal of Central European Agriculture, 18(1), 55-72.*
10. *Sandhya, R. 2016. Development of nutrient seed coating formulation to improve seedling vigour of cotton and maize. M. Sc.(Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.*
11. *Sharma, S, Jasjeet K. Sahni, Javed Ali and Sanjula Baboota (2015). Effect of high-pressure homogenization on formulation of TPGS loaded nanoemulsion of rutin - pharmacodynamic and antioxidant studies. Drug Deliv, 22: (4) 541-551.*
12. *Singh, D., & Sharma, N. (2017). Effect of chromium on seed germination and seedling growth of green garm (Phaseols Aureus L) and chickpea (Cicer Arietinum L). Int J App Nat Sci, 6, 37-46.*
13. *Sridhar, C. 2012. Effect of nanoparticles for the maintenance of tomato seed vigour and viability. M.Sc. Thesis, Tamil Nadu Agricultural University, Coimbatore.*

APPENDIX

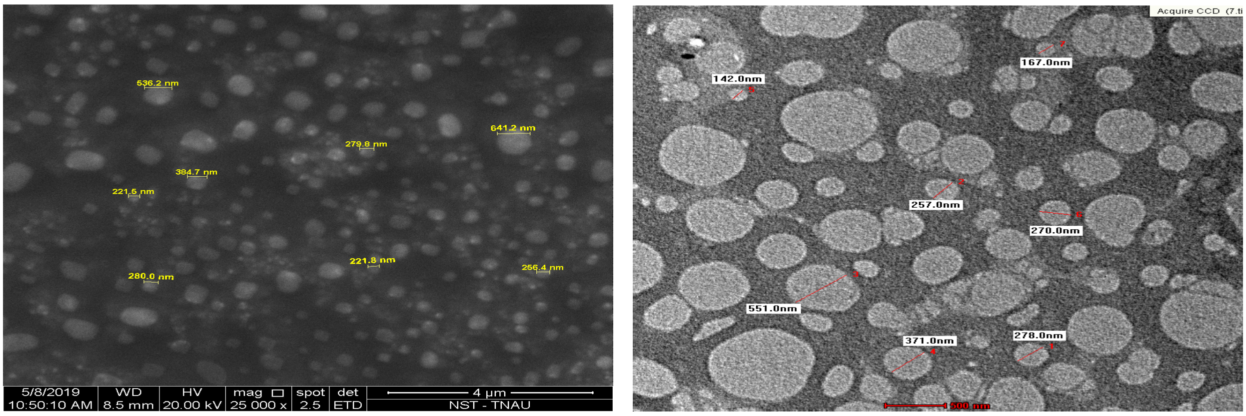

Figure 1: SEM and TEM Images of Methyl Cellulose based Vitamin E Loaded Nano Formulation

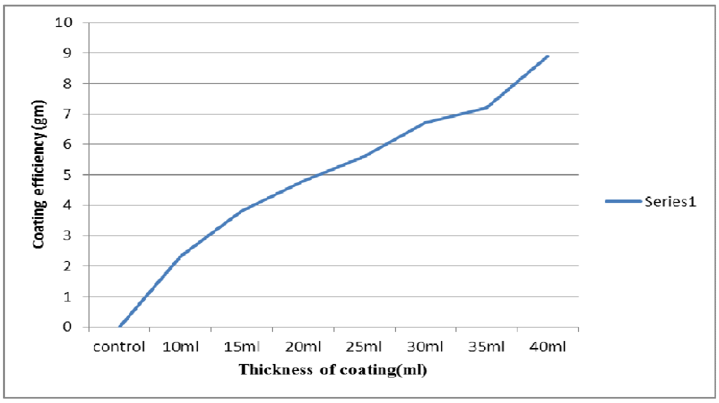

Figure 2: Coating Efficiency of Methyl Cellulose Based Vitamin E Loaded Nano Formulation

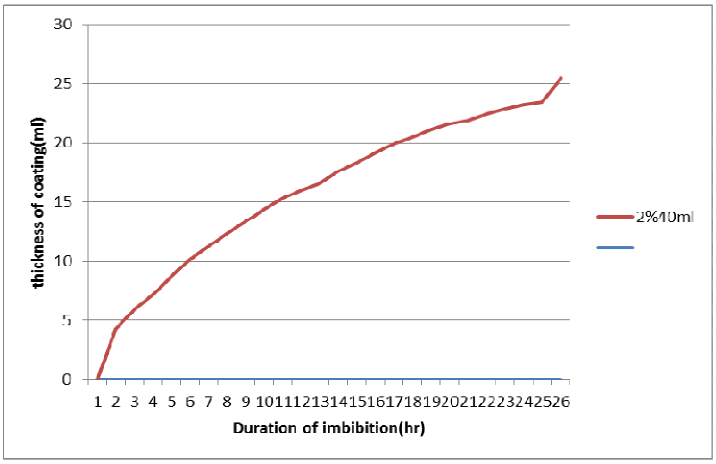

Figure 3: Imbibition Rate Effect of Methyl Cellulose based Vitamin E Loaded Nano Formulation (40ml/ kg of Seed)

Table 1: Effect of Methyl Cellulose based Vitamin E Loaded Nano Emulsion on Maize Seedlings

Coating Thickness (ml/kg)	Physiological Parameters					
	Speed of Germination (%)	Germination (%)	Root Length (cm)	Shoot Length (cm)	Dry Matter Production (gm/10seedling)	Vigour Index
Control	7.4	96 (78.46)	18.6	12.3	0.954	2966
10ml	7.9	97 (80.02)	20.4	14.7	1.053	3440
15ml	7.8	98 (81.87)	21.5	13.9	1.073	3540
20ml	8.1	100 (89.71)	21.4	13.9	1.143	3530
25ml	7.9	100 (89.71)	21.3	14.6	1.147	3590
30ml	8.0	98 (81.87)	21.7	15.2	1.178	3616
35ml	8.1	98 (81.87)	21.8	16.0	1.223	3704
40ml	8.2	100 (89.71)	22.1	16.3	1.250	3840
Mean	7.9	98 (81.87)	21.1	14.61	1.127	3528
SEd	0.14	2.3	0.54	0.339	0.0206	59.0
CD (P=0.05)	0.31	4.9	1.15	0.720	0.0438	125.1

(Figure in parenthesis indicate arcsine values)